J. Clin. Chem. Clin. Biochem.
Vol. 19, 1981, pp. 529–538

Evolution of Clinical Enzymology[1])

By *J. Büttner*

Institut für Klinische Chemie der Medizinischen Hochschule Hannover

(Received March 31/July 11, 1980)

Herrn Prof. Dr. H. U. Bergmeyer zum 60. Geburtstag gewidmet

Summary: The evolution of clinical enzymology is discussed in relation to the history of general enzymology and clinical chemistry. The discussion is limited to the period from 1835 (definition of catalysis by *Berzelius*) to 1935 (description of the optical test by *Warburg*). In conclusion, a general account is given of the introduction of the concept of quantitative enzyme activity determination into clinical medicine.

Die Entwicklung der klinischen Enzymologie

Zusammenfassung: Die Entwicklung der Klinischen Enzymologie wird vor dem Hintergrund der Geschichte der Allgemeinen Enzymologie sowie der Klinischen Chemie dargestellt. Die Schilderung beschränkt sich auf den Zeitraum von 1835 (Definition des Begriffes Katalyse durch *Berzelius*) und 1935 (Beschreibung des optischen Tests durch *Warburg*). Abschließend wird die Rezeption des Konzeptes quantitativer Enzymaktivitätsbestimmungen durch die Klinische Medizin unter allgemeinen Gesichtspunkten besprochen.

Introduction

Hugo Theorell, at the 3rd International Congress of Clinical Chemistry in Stockholm 1957, remarked on the use of enzyme reactions in clinical chemistry: ". . . The development has been rather slow until a few years ago; if I may borrow an expression from bacteriology we may say that it remained in its "lag phase" for many years. Now we have obviously come to the "log phase" (1)". This remark characterizes very well the development of clinical enzymology, which today, after a truly logarithmic growth, plays a central part within clinical chemistry. The following is an attempt to describe the historical development of clinical enzymology. I shall confine myself to that "lag phase", which is approximately the period from 1835 to 1935, marked by *Berzelius*' definition of catalysis (2) and by *Warburg*'s work on cell enzymes (3).

Milestones in the History of Enzymology

First it would be desirable to cast a glance at the history of general enzymology. Various descriptions by competent authors are available on this subject (4). So it will suffice here to recall some of the milestones of development.

The problems of fermentation had been studied since the antiquity. But only the revolution of chemistry, initiated by *Lavoisier* and his contemporaries, gave prominence to the anomaly of such processes which did not seem subject to the laws of stoichiometry.

With the farsighted creation by *Berzelius* of the term catalysis (1836) (2), there emerged a new paradigm as defined by *T. S. Kuhn* (5). Table 1 summarizes the experimental facts that *Berzelius* proceeds from. *Liebig*

Tab. 1. *Berzelius* (1835) "catalytic force" and "catalysis".

Definition (2)	
"Materials act by their mere presence . . . not on account of their chemical affinities . . . without necessarily participating in the reaction".	
Experimental facts	
C. G. S. Kirchhoff (1812)	Acid hydrolysis of starch (67)
H. Davy (1817)	Oxidation of alcohol and ether by platinum (68)
L. J. Thenard (1818)	Decomposition of H_2O_2 (69)
J. W. Döbereiner (1823)	Ignition of H_2 in air by platinum (70)
E. Mitscherlich (1834)	Formation of ether (71)

[1]) Presented at the Symposium on History of Clinical Chemistry, 3rd European Congress of Clinical Chemistry, Brighton, 6-6-1979.

0340-076X/81/0019-0529$02.00

& *Wöhler*, in their description of emulsin, which they isolated in the following year, made express reference to *Berzelius*' definition (6). Shortly before, the investigation of the digestive process had been initiated by the studies of *Tiedemann & Gmelin* (1824) as well as *Eberle* (1834). In 1836 *Theodor Schwann* succeeded in isolating the first digestive enzyme, pepsin (7) (tab. 2). Alcoholic fermentation, too, seemed to fit into the pattern created by *Berzelius*. By the virtually concurrent yet independent works (8) of engineer *Cagniard de Latour*, botanist *Kützing*, and physiologist *Schwann*, yeast was recognized, in 1837, as a living vegetable organism. There will be no fermentation of sugar solutions if the access and development of "Zuckerpilz" (*Schwann* (9)) is prevented by suitable measures.

These findings gave rise to vehement discussions which continued over the following 35 years and in which *Louis Pasteur* intervened from 1837 on (10): On the one side, the reductionistic thesis, argued mainly by *Liebig* (tab. 3), of the chemical action of ferments – on the other side the thesis, supported with ingenious experiments by *Pasteur*, that the action of fermentation is produced by living cells. This argument, known as the "*Liebig/Pasteur* Controversy", certainly delayed the investigation especially of "soluble ferments" or enzymes, a term coined by *Kühne* in 1877.

Tab. 2. Early fundamental work on digestion.

1826	*F. Tiedemann & L. Gmelin* (72) Die Verdauung nach Versuchen
1833	*W. Beaumont* (73) Experiments and observations on the gastric juice . . .
1834	*J. N. Eberle* (74) Physiologie der Verdauung, nach Versuchen . . .
1836	*Th. Schwann* (75) Über das Wesen des Verdauungsprozesses (discovery of pepsin)
1846	*F. Th. Frerichs* (76) Die Verdauung
1852	*F. Bidder & C. Schmidt* (58) Die Verdauungssäfte und der Stoffwechsel

This stage of development ended with another change of paradigm: In 1896 *Eduard Buchner* detected fermentation by cell-free yeast press juice (11). Meanwhile

Tab. 3. Early theories of enzyme action.

1839	*Liebig* (72) (and *Nägeli* 1879 (78)) Decomposing ferments act mechanically (state of inner movement) on other substances
1858	*M. Traube* (79) Activation of molecular oxygen
1894	*E. Fischer* (80) Specificity, theory of "lock and key"
1902	*W. Ostwald* (59) Enzymes as "biocatalysts"
1903	*Henri* (62)
1913	*Michaelis & Menten* (63) Intermediate complexes with substrate

there were the first signs of a theory of enzyme action (tab. 3). By the end of century, only a relatively small number of enzymes had been isolated and more closely characterized (tab. 4), so that the chemical structure of enzymes, i.e. their protein nature, was still a matter of speculation. The proof of this was furnished as late as in 1926, by *Sumner*'s crystallization of urease (12), a discovery which introduces the era of modern enzymology.

Tab. 5. Development of clinical chemistry. (underlined: pupils of *J. Liebig*).

Phase	Characteristics	Figures	
Early phase 1840–1860 (1880)	Elementary analysis. Animal chemistry (concept of *Liebig* 1842)	*Simon* *Rees* *Bence-Jones* *Heller* *Scherer* *A. Becquerel*	*Golding Bird* *J. Vogel* *Pettenkofer* *C. Schmidt* *Hoppe-Seyler* *Thudichum*
Foundation of clinical laboratories 1880–1932	Development of methods, micromethods	*Jaffé* *Salkowski* *Folin*	*Bang* *Rona*
Beginning of independence since 1932	Colorimetry, photometry. Enlarged program: enzymes, hormones and others	*van Slyke* and many others	

Tab. 4. Important enzymes known in 19th century.

Name	Discovery	Isolation	Crystallization
Diastase	*Kirchhoff* 1814 (81)	*Payen & Persoz* 1833 (82)	*Meyer* et al. 1948 (83)
Pepsin	*Eberle* 1834 (74)	*Schwann* 1836 (75)	*Northrop* 1930 (84)
Emulsin	*Roubiquet* et al. 1830 (85)	*Liebig & Wöhler* 1837 (6)	–
Invertase	*Dubrunfaut* 1846 (86)	*Berthelot* 1860 (87)	–
Urease	*Fourcroy & Vauquelin* 1799 (20)	*Musculus* 1876 (88)	*Sumner* 1926 (12)
Trypsin	*Corvisart* 1857 (89)	*Kühne* 1877 (90)	*Northrop* et al. 1931 (91)
Papain	*Hughes* 1750 (92)	*Wurtz* et al. 1879 (93)	*Balls* et al. 1939 (94)

Main Dates of the Evolution of Clinical Chemistry (13)

Before we discuss the development of clinical enzymology, it is necessary to give a brief outline of the essentials of the *history of clinical chemistry*, in order that clinical enzymology may be seen in the context of the historical framework thus created. The central idea of clinical chemistry, to use changes in the composition of body materials to diagnose diseases, can be traced far back in the history of medicine. Appropriate analytical methods were lacking for a long time. *Robert Boyle* (1627–1691) in 1684 was the first to develop a program for chemical analysis of blood but it took more than 150 years to get appropriate analytical methods for this task. Based on the work of *A. L. Lavoisier* (1743–1794), systematic quantitative analysis of biological materials ("animal chemistry") was started by *Fourcroy* (1755–1809) and *Berzelius* (1779–1848), improved by *Gay-Lussac* (1778–1850) and *Thénard* and finally perfected by *Justus Liebig* (1803–1873).

During the third decade of the 19th century the analytical tools had become available, but doctors were – for several reasons – not interested in the broad application of chemical analysis in practical medicine.

The empirical medicine of the French School, based on immediate observation, used percussion, auscultation and anatomic examination, but chemical analysis was largely absent from diagnosis.

In Germany the situation was quite different due to the great influence of *Schelling*'s "Naturphilosophie". *Schelling* was not antipathetic to chemistry, but trying to compass all natural phenomena as a whole, he was more interested in principles than in experimental details. The medical concepts of this period were speculative, without close relation to medical practice. There was no place for results of chemical analyses in clinical diagnostics.

The swing to the opposite direction – and therefore the start of clinical chemistry – was brought about mainly by *Justus Liebig*'s monograph "Die organische Chemie in ihrer Anwendung auf Physiologie und Pathologie", in April 1842. This book was of particular significance for the development of clinical chemistry as it introduced a quantitative method of observation into physiological chemistry and therefore encouraged doctors also to apply quantitative analysis to the diagnosis of diseases (14). In the meantime a new scientifically oriented clinical medicine had started to develop, first of all in France, where the term "physiological medicine" was coined for it. This way of thinking was taken up in England, Austria, and finally in Germany. Young doctors and chemists – many of them pupils of *Liebig* – started chemical investigations in hospitals (tab. 5).

From a clinical point of view, the results were not very convincing, the impact on practical medicine was small, so basic research came more and more into the foreground from about 1860 onward. It is only around 1880 that clinical chemistry was revived; clinical laboratories then became established and the methodics of the discipline were expanded systematically.

The stage of independence as a discipline began around 1932 with the publication of the major monograph by *Peters & van Slyke* (15). Increasingly, patho-biochemistry, too, took its place alongside the development of methods, although it was only after the second World War that the number of organizationally independent clinical chemical laboratories increased world-wide.

Evolution of Clinical Enzymology (16)

Within the historical context already described, how has clinical enzymology evolved?

The concept of using *enzymes as specific reagents* for clinical chemical analyses can be traced far back. In 1780 *Francis Home*, Professor of materia medica at Edinburgh, described the fermentation test with yeast for the detection of glucose in urine (17), a process that has seen many modifications (e.g. the *Einhorn*-Saccharimeter (18), (fig. 1)) and has been used until recently. *Carl Schmidt*, in 1850, on the basis of this principle, carried out very exact determinations of glucose in blood (19); it then became possible to determine fasting blood sugar quantitatively for the first time.

In the examination of urine, the processes of decomposition (called *alkaline and acid fermentation of urine*)

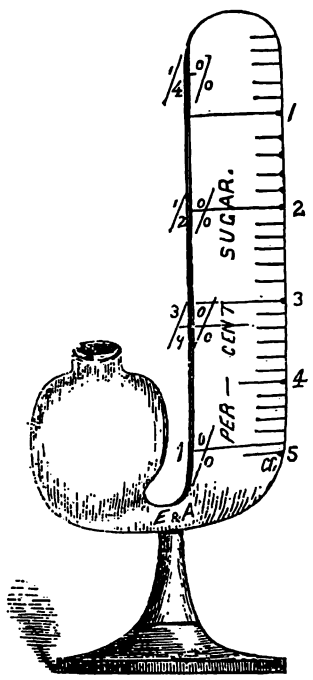

Fig. 1. *Einhorn*-Saccharimeter (from the original publication (18)). The right part is filled with a mixture of urine and ca. 1 g of yeast. After 24 h the CO_2 volume shows the glucose content.

attracted early attention. *Fourcroy & Vaquelin* (20), in 1799, were right in interpreting "alkaline decomposition" of urine as a fermentative decomposition of urea. Only a short time after *Schwann*'s experiments on fermentation, these processes were shown to be associated with yeast cells identified in microscopic sediment analyses of decomposing urine samples. In 1860, *Pasteur* succeeded in proving that bacteria are the cause of the alkaline fermentation of urine (21).

The concept, so familiar to us today, of determining *enzyme activities in the blood of patients*, was expressed for the first time by *Carl Schmidt*, a physiological chemist of Dorpat (1822–1894) (22). *Schmidt* was a pupil of *Liebig*, *Wöhler* and *Rose* and later became the teacher of *Wilhelm Ostwald.* In 1850, he published a work on transsudation anomalies (19), fundamental for early clinical chemistry, in which he reported on some quantitative tests for the detection of enzymes in blood (tab. 6) (22). The results of these experiments, however, were rather poor as the occurrence of bacterial decomposition led to uncontrolled variations.

Enzyme activities in digestive juices would have been an obvious choice for investigation after the major works on the physiology of digestion (tab. 2). Yet clinical chemistry was rather hesitant in this area around the mid-19th century. Although the leading text-books of the new discipline "Physiological Chemistry", very soon dealt with digestion at some length (23), decades had to pass before the diagnostic analyses of digestive juices were employed in clinical medicine. Methods of clinical chemical examination for the enzymes of the gastric and pancreatic juices are first given around 1860, e.g. by *Felix Hoppe (-Seyler)* in his "Anleitung zur pathologisch-chemischen Analyse" (24). *Wilhelm Valentiner* placed more emphasis on the clinical aspects. As an assistant under *v. Frerichs* in Breslau and Berlin, *Valentiner* had learnt how to apply chemical methods in diagnostics (25). At first, broader application was faced with practical difficulties such as the obtaining of gastric juice; the use of emetics was the only method. With the introduction of the stomach tube (by *Leube* 1871 (26)) for diagnostic purposes, determinations of enzymes, for the first time, met with greater interest in clinical medicine. These methods were covered in detail by the textbooks on clinical diagnostics, which began to appear from about 1890 (see l.c. (27)). As an example it suffices to mention the well known German book by *Sahli*, Lehrbuch der Klinischen Untersuchungsmethoden (27). These books show, on the other hand, that the *reception of the enzyme concept* by the clinicians is restricted to digestive enzymes, which are primarily accessible to the physician.

Tab. 6. *Carl Schmidt* 1850.
Charakteristik der epidemischen Cholera gegenüber verwandten Transsudationanomalieen, Leipzig und Mitau (19), pp. 57ff.

Typical experiment			
1 ml blood	1 ml blood	1 ml blood	1 ml blood
0.5 g glucose	0.2 g urea	0.1 g amygdalin	0.1 g asparagin
4 ml water	4 ml water	4 ml water	4 ml water
Incubation at room temperature for about 10 days			
Observation of production of			
CO_2	NH_3/ $(NH_4)_2CO_3$	HCN	NH_3/ $(NH_4)_2CO_3$

From the clinical literature of this period it becomes clear that the concept of catalysis was not accepted by the clinicians. As late as 1907, the efforts of *Heinrich Schade* (1876–1935), later well known for his work on physical chemistry in internal medicine, to introduce the concept of catalysis in clinical medicine (28), met with complete lack of comprehension. The medical faculty of Kiel University did not accept his book on catalysis in medicine (29).

In the meantime various *enzymes* were detected *in blood and urine* (30), the usual materials for clinical chemical examinations. However, the available methods for determination were much to intricate for clinical use.

In 1910, *Julius Wohlgemuth*, then at the experimental-biological department of the Pathological Institute in Berlin, proposed a method, worked out by himself a short time previously, for the determination of amylase (diastase) in blood and urine for pancreas diagnostics. He had observed that an occlusion of the ductus pancreaticus, both experimentally in animals and in the patient, results in a strong increase of amylase in the urine. He says: "After these findings I think it is clearly indicated that in all future cases with suspected occlusion of the pancreatic duct, the method I have described should be used to examine the urine for its diastase content" (31).

Wohlgemuth's method with certain modifications, was still in use in the nineteen-fifties. We should add that even now we have not quite reached that target of an advanced standardized method for amylase. Shortly afterwards, the determination of lipase in the serum was added to that of amylase. In 1911, *Peter Rona* (1871–1949), then head of the chemical department at the hospital "Am Urban" Berlin (32) had developed, together with *Leonor Michaelis*, a stalagmometric method for lipase determination (33) (fig. 2). It is worth noting that the inhibitors of this enzyme (atoxyl and quinine) were already employed by *Rona* (34) for the differentiation of lipases from different organs ("atoxyl-resistant pancreatic lipase").

Rona says in this connection that one should "try to use this approach for the stepwise elaboration of a kind of ferment topography, i.e. the determination of the

origins of individual types of ferments in a mixture of ferments" (35).

The clinical use of amylase and lipase determinations for the diagnostics of pancreatic diseases was strongly advocated by the German clinician *Gerhard Katsch* from around 1924 (36). It was he who coined that easily remembered term, "Fermententgleisung" (enzyme derailment), for the transition of pancreatic enzymes into the blood (28).

A new chapter of clinical enzymology was opened with *Archibald Edward Garrod*'s epoch-making concept of the "inborn errors of metabolism", which he presented in the Croonian Lectures in 1908 (37). However, a further 40 years were to elapse before the first enzyme defect of this kind was actually proved (38). An important enzymological discovery initiated the evolution of another extensive and clinically important field of work: In 1872, *Alexander Schmidt*, in Dorpat, detected the "*fibrin ferment*", later called thrombin (39), thereby opening the way to the explanation of the coagulation process. But apart from the bleeding and coagulating times, it was only the determination of prothrombin time, described by *Quick* (40) in 1935, that was used clinically.

Another stimulus for clinical enzymology came from quite a different quarter (tab. 7): In 1923, *Robert Robison* (1883–1941) had detected a *phosphatase* which splits monoesters of phosphoric acid, and which is present to a large extent in bone (41). Subsequent to this discovery, he developed a theory of ossification (42), which attracted immediate attention. In 1929, *H. D. Kay*, who had previously worked under *Robison*, reported an increase of phosphatase in blood plasma in Osteitis deformans (43), and he described a quantitative method for determining this enzyme, based on the determination of the phosphate released from β-glycerophosphate (44). This method was subsequently improved and also found to be relevant to the diagnostics of liver and bile duct diseases (45). At first, the method appeared too complicated from the clinical point of view (46), and was accepted rather hesitantly by clinical laboratories; strictly speaking, it was not widely used until after the second World War. One should not forget, on the other hand, that this was the first instance where methods for the determination of alkaline phosphatase were set up according to advanced criteria which are valid even today, i.e. as regards the constancy of the conditions of reactions and the use of the chemically defined substances.

Careful research of the pH optimum of the various phosphatases very soon resulted in the delineation of a specific *acid phosphatase* in the prostate gland (47) (tab. 8). *Aaron B. Gutman & Ethel B. Gutman* (48) showed, in 1936, that this acid prostatic phosphatase is also produced in the cells of a prostatic carcinoma and, in 1938 they demonstrated the transition of this enzyme into the blood; they therefore succeeded in developing the first enzymatic method for the diagnosis of carcinoma (49). Thus another enzyme activity determination was introduced to clinical medicine.

Between the first and second World Wars, numerous attemps were made to use further enzymes for clinical diagnostics (cf. l.c. (50)). The results of these efforts were largely unsatisfactory; this was due partly to controversies concerning clinical usefulness, e.g. of the "Abwehrferments" described by *Abderhalden* (51).

The turning point and the beginning of the present era of clinical enzymology is marked by a publication which came out during the second World War and, because of the war, was hardly taken notice of at the time. In 1943, *Otto Warburg & Walter Christian* published a paper on

Tab. 7. Clinical enzymology of alkaline phosphatase.

Early development		
1922	*R. Robison* (41)	*Robison*-ester
1923	*R. Robison* (42)	Bone phosphatase, theory of ossification
1929	*H. D. Kay* (43)	Plasma phosphatase in bone diseases
1930	*W. M. Roberts* (45)	Plasma phosphatase in liver-bile diseases
Activity determination of alkaline phosphatase (44, 45)		
1930	*Kay*	(β-glycerophosphate, pH 7.6)
1933	*Bodansky*	(β-glycerophosphate, pH 8.6)
1934	*King, Armstrong*	(phenylphosphate, pH 9.3)
1945	*Huggins, Talalay*	(phenolphthalein phosphate, pH 9.1–9.6)
1946	*Bessey, Lowry, Brock*	(*p*-nitrophenylphosphate, pH 10.3)

Tab. 8. Clinical enzymology of acid phosphatase.

Early development		
1934	*Bamann, Riedel* (47)	Phosphatases with different pH optimum
1935	*Kutscher, Wolbergs* (47)	High acid phosphatase activity in prostate
1936	*Gutman, Sproul, Gutman* (48)	Synthesis of acid phosph. tase in prostatic carcinoma cells
1938	*Gutman, Gutman* (49)	Acid phosphatase in plasma increased in prostatic carcinoma
Activity determination of acid phosphatase		
1938/40	*Gutman, Gutman* (95)	(phenylphosphate, pH 4.9)
1945	*Huggins, Talalay* (45)	(phenolphthalein phosphate, pH 5.75)
1947/48	*Abul-Fadl, King* (96)	(inhibition of prostatic enzyme by *L*-tartrate)
1953	*Fishman, Lerner* (97)	(specific determination of prostatic enzyme by means of *L*-tartrate)

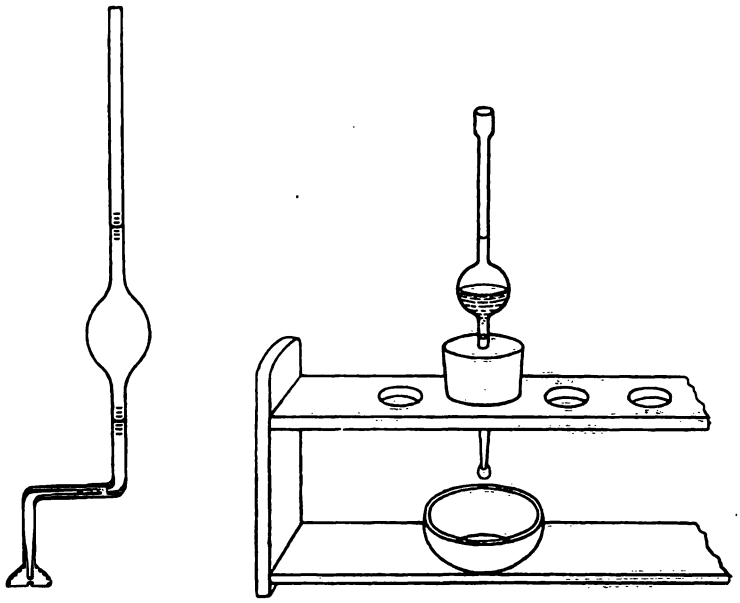

Fig. 2. Stalagmometer used for lipase activity determination (from l.c. (66)). Lipase action on tributyrin reduces surface tension, measured as drops/min. a: after *J. Traube*; b: after *Rona & Michaelis*.

enzymes of glycolysis in the blood serum of tumour rats (3). This followed the development, during the preceding years, of the necessary methodical tools, in particular the "optical test" for the kinetic determination of enzyme activities (3).

Determination of Enzyme Activities as a Quantifying Diagnostic Method

At this point, I propose not to follow up the rapid development, which set in after the second World War, and which has led to the prominent position of enzyme activity determination in today's clinical chemistry. Instead it would seem worthwile to consider more general aspects and to look into the question of how the concept of *quantitative* measurement of enzyme activity developed and how it found acceptance in the world of clinical medicine.

Rothschuh & Bleker (52) have pointed out that scientific measuring methods were not introduced into clinical diagnostics until about the 2nd half of the 19th century; and this mainly under the influence of the physical school of thought in physiology, evolving from 1840 and marked by names such as *Brücke*, *Helmholtz*, *du Bois-Reymond*, *Ludwig*, and *Vierordt*. We should mention here, e.g. the measurement of pulse frequency, blood pressure, body temperature; also the methods of spirometry and blood cell counting, and the quantitative techniques of ophthalmology.

At that time, considerable difficulties, both theoretical and practical, were still standing in the way of quantifying enzyme activities for clinical purposes.

First it was necessary to define the term "enzyme activity" exactly. *Berzelius* (2), in 1836, had spoken of "Katalytische Kraft" (catalytic force), a term to be found in textbooks even after the second World War, whereas *Schwann* (53) coined the term "metabolische Kraft" (metabolic force). In *Hoppe-Seyler*'s textbook of 1883 (54) one even finds "Energie der Verdauung" (energy of digestion). Such rather vague terminology was bound to make impossible an adequate definition of a system of measurement.

It is worth noting that around the mid 19th century there was still a complete lack of tools on the part of chemistry. Chemists, in the words of *Edmund Farber* (55), "were late in developing time-consciousness". After *Wilhelmy*'s classic work about the law of time in cane sugar inversion by acids (1851) (56), though unnoticed at the time, it was *M. Berthelot* (1862) (57) who first studied the measurement of reaction velocities. But it was the newly developing physical chemistry, under *Ostwald*, *van't Hoff*, *Arrhenius*, and others, that produced a kinetic theory of reactions, thus also creating the basis for the study of catalytic and enzymatic reactions.

Meanwhile, in connection with the study of "digestive ferments", the first experiments had been carried out on the quantification of enzyme action. Such experiments were needed for a comparison between different enzyme preparations (e.g. pepsin (7)).

The most careful experiments in the early days were undertaken by *Bidder & Schmidt* in Dorpat (1852) (58), who used cylinders of coagulated egg albumin, and determined their decrease in weight under the influence of pepsin. The principle of series dilution of enzyme solutions became widely used; for instance, in *Wohlgemuth*'s method, mentioned earlier, for diastase determination (31).

Initially, the study of the kinetics of enzyme reactions produced inconsistent results. This was discussed by *Wilhelm Ostwald* in his paper "Über Katalyse" at the 1901 "Naturforscherversammlung" (59). Thus, certain studies gave rise to doubts whether enzyme reactions were, like other catalytic reactions, subject to the law of mass action.

O'Sullivan & Tompson (60), *A. Brown* (61), *Henry* (62), and finally *Michaelis & Menten* (63), in a series of very careful studies on invertase, whose activity was easy to monitor polarimetrically, succeeded in deriving exact rate equations, which ultimately led to the really constructive concept of the enzyme-substrate intermediate.

It then also became possible to define quantities for enzyme activity based on enzyme kinetic measurements. Initially, however, these were only used for a characterization of purified enzyme preparations. For clinical enzyme activity determinations, recourse was taken to a definition of arbitrary units, which quickly grew into an immense variety. Worldwide standardization was reached as late as in 1961, when the International Enzyme Unit was introduced (64).

The importance of test conditions for the measuring of enzyme activities had meanwhile become apparent. One had learnt to define the reaction temperature, pH, substrate concentration, etc., as the basis for achieving consistent results. The work of *Michaelis & Menten* (63) had proved that it was possible to measure enzyme activities with an excess of substrate as zero-order reactions at maximum reaction velocities. Finally, from 1935 onwards, with the development of the universally applicable principle of the optical test by *Otto Warburg* (3), and with the advent of suitable photometers, the theoretical and practical requirements existed for using that complex measuring quantity, "enzyme activity", in clinical chemistry; that is, for carrying out determinations of enzyme activities in addition to the traditional determinations of concentrations.

Widespread reception by the clinical world became possible from about the end of the second World War, after workable pathophysiological concepts had become available for clinical use; e.g. for pancreatic enzymes, phosphatases, cell enzymes.

Robert E. Kohler (65) has pointed out that modern dynamic biochemistry came into being at the same time as, and in connection with, enzymology (or the enzyme theory or life). To me it would seem that a similar connection exists between modern clinical chemistry and clinical enzymology. Clinical chemistry has discovered the "dimension of time"; it has acquired an aspect of dynamics, following the evolution of clinical enzymology in its midst.

Reference and Notes

1. Theorell, H. (1958), Scand. J. Clin. Lab. Invest. *10*, Supplementum *31*, 91.
2. Berzelius, J. (1836), Einige Ideen über eine bei der Bildung organischer Verbindungen in der lebenden Natur wirksame, aber bisher noch nicht bemerkte Kraft.
   In: Jahresbericht über die Fortschritte der physischen Wissenschaften *15*, 237–245.
   This report was presented to the Swedish Academy of Science by *Berzelius* on 31. 3. 1835, the German translation was published in 1836. See: Berzelius, J. J., (1837), Lehrbuch der Chemie, 3. Auflage, Vol. *6*, 19–25, Arnoldi, Dresden und Leipzig.
3. Particularly *Warburg*'s papers on the 'optical test' (Warburg, O., Christian, W. & Griese, A. (1935), Biochem. Z. *282*, 157) and on enzymes in blood of tumour rats (Warburg, O. & Christian, W. (1943), Biochem. Z. *314*, 399).
4. For larger reviews on the history of enzymology see: Mittasch, A. (1939), Kurze Geschichte der Katalyse in Praxis und Theorie; Springer-Verlag, Berlin.
   Walden, P. (1949), Aus der Entwicklungsgeschichte der Enzymologie von ihren Anfängen bis zum Ausbruch des zwanzigsten Jahrhunderts. In: Ergeb. Enzymforsch. *10*, 1–64.
   Schadewaldt, H. (1966), Zur Geschichte des Fermentbegriffes; In: Festschrift der Kali-Chemie AG, Hannover.
   Keilin, D. (1970), The History of Cell Respiration and Cytochrome; University Press, Cambridge.
   Dixon, M. (1970), The History of Enzymes and of Biological Oxidations; In: The Chemistry of Life (Needham, J., ed.), University Press, Cambridge.
   Fruton, J. S. (1972), Molecules and Life; Wiley Interscience, New York–London–Sydney–Toronto.
5. Kuhn, T. S. (1962), The Structure of Scientific Revolutions; University of Chicago, Chicago.
6. Liebig, J. & Wöhler, F. (1837), Über die Bildung des Bittermandelöls; Liebigs Ann. Chem. Pharm. *22*, 1.
7. Hickel, E. (1975), Pepsin, ein Veteran der Enzymchemie. Naturwiss. Rundsch. *28*, 14–18.
   Mehlan, W. (1976), Das Werk von Friedrich Tiedemann und Leopold Gmelin "Die Verdauung nach Versuchen"; Inaug. Dissertation, Bonn 1976.
8. See: Keilin, D. (1970), l. c. (4).
9. Schwann, Th. (1837), Vorläufige Mittheilung, betreffend Versuche über die Weingährung und Fäulniß; Liebigs Ann. Chem. Pharm. *41*, 184–193.
10. For details see:
    Finegold, H. (1954), The Liebig-Pasteur Controversy; J. Chem. Educ. *31*, 403–406.
    Keilin, D. (1970), l. c. (4).
    Doke, T. (1971), The Controversy between J. Liebig and L. Pasteur; 12th Inter. Congress of History of Sciences, Paris, 1968, Actes *8*, 31–39.
11. Buchner, E. (1897), Alkoholische Gärung ohne Hefezellen (Vorläufige Mitteilung): Ber. Dtsch. Chem. Ges. *30*, 117–124.
    Buchner was awarded the Nobel Price for medicine and physiology 1907. See the important papers by R. E. Kohler on this topic:
    Kohler, R. E. (1971), The background of Eduard Buchner's discovery of cell-free fermentation. J. Hist. Biol. *4*, 35–61.
    Kohler, R. E. (1972), The reception of Eduard Buchner's discovery of cell-free fermentation. J. Hist. Biol. *5*, 327–353.
12. Sumner, J. B. (1926), J. Biol. Chem. *69*, 435.
13. On development of clinical chemistry:
    Büttner, H. (1977), Geschichte der Klinischen Chemie; Med. Welt (N. F.) *38*, 1238–1243.
    and: Büttner, J. (1978), Johann Joseph von Scherer (1814–1869). Ein Beitrag zur frühen Geschichte der Klinischen Chemie. This J. *16*, 478–483.
14. Liebig, J. (1842), Die organische Chemie in ihrer Anwendung auf Physiologie und Pathologie. Vieweg, Braunschweig.

See especially the introduction to the facsimile edition of the English translation by Holmes, F. L. (1964), Johnson Reprint Corporation; New York and London, VII–CXVI.

15. Peters, J. P. & van Slyke, D. D. (1931/32), Quantitative Clinical Chemistry; 2 vols., Williams and Wilkins, Baltimore.
16. Many details of the history of clinical enzymology are included in the monograph by Richterich, R. (1958), Enzympathologie. Enzyme in Klinik und Forschung; Springer-Verlag, Berlin, Göttingen, Heidelberg.
See particularly: the recent paper by Habrich, C. (1978), Zur Geschichte der Klinischen Enzymdiagnostik und ihren wissenschaftlichen Voraussetzungen. In: Medizinische Diagnostik in Geschichte und Gegenwart.
Festschrift Heinz Goerke. (Habrich, C., Marguth, F., Wolf, J. H. & Fritsch, W., eds.) München, pp. 549–571.
17. Home, F. (1780), Clinical experiments, histories and dissections; Edingburgh.
18. The *Einhorn* Saccharimeter was proposed by Einhorn, M. (1887), New York Medical Recorder *1887*, 91 (see fig. 1).
19. Schmidt, C. (1850), Charakteristik der epidemischen Cholera gegenüber verwandten Transsudationsanomalieen, G. A. Reyher, Leipzig und Mitau.
20. Fourcroy, A. F. & Vauquelin, N. T. (1799), Pour servir à l'histoire naturelle chimique et médicale de l'urine humaine, Ann. Chim. *31*, 48–71 (see ibid. *32* (1800) 80).
21. Pasteur, L. (1860), De l'origine des ferments. Nouvelles expériences relatives aux générations dites spontanées. C. R. Acad. Sci. (Paris) *50*, 849–54.
22. *Carl Schmidt*'s early work on enzyme determinations was recently pointed out by C. Habrich (l. c. (16)). For a biography of *Carl Schmidt* see
Zaleski, S. S. (1894), Carl Schmidt, Ber. Dtsch. Chem. Ges. *27*, 963–978, and
Bing, F. C. (1973), Friedrich Bidder (1810–1894) and Carl Schmidt (1822–1894) – A Biographical Sketch, J. Nutrition *103*, 639–648 (with Portrait).
23. For example:
Lehmann, C. G. (1850/52), Lehrbuch der physiologischen Chemie, 3 vols, W. Engelmann, Leipzig.
Kühne, W. (1866/8), Lehrbuch der physiologischen Chemie, W. Engelmann, Leipzig.
Hoppe-Seyler, F. (1877/81), Physiologische Chemie; 4 parts in 2 vols., A. Hirschwald, Berlin.
Bunge, G. (1887), Lehrbuch der physiologischen und pathologischen Chemie. F. C. W. Vogel, Leipzig.
24. Hoppe, F. (1858), Anleitung zur pathologisch-chemischen Analyse für Aerzte und Studirende, A. Hirschwald, Berlin.
25. Valentiner, W. (1863), Die Chemische Diagnostik in Krankheiten, F. Schneider, Berlin.
26. *Leube, W.*, had reported on diagnostic use of stomach tube at the 1871 meeting of the Gesellschaft Deutscher Naturforscher und Ärzte at Rostock;
see: Leube, W. (1883), Beiträge zur Diagnostik der Magenkrankheiten, Dtsch. Arch. Klin. Med. *33*, 1.
27. Seifert, O. & Müller, F. (1886), Taschenbuch der Medizinisch-klinischen Diagnostik, J. F. Bergmann, Wiesbaden.
Wesener, F. (1890), Lehrbuch der chemischen Untersuchungsmethoden zur Diagnostik innerer Krankheiten, F. Wreden, Berlin.
Lenhartz, M. (1893), Mikroskopie und Chemie am Krankenbett, J. Springer, Berlin.
Sahli, H. (1894), Lehrbuch der klinischen Untersuchungsmethoden, F. Deuticke, Leipzig und Wien.
Geigel, J. & Voit, F. (1895), Lehrbuch der klinischen Untersuchungsmethoden, F. Enke, Stuttgart.
28. Schade, H. (1907), Die Bedeutung der Katalyse für die Medicin, W. G. Mühlau, Kiel.
29. Hadjamu, J. (1974), Prof. Dr. med. Heinrich Schade. Begründer der Molekularpathologie, 1876–1935. Leben und Werk. Düsseldorf (Düsseldorfer Arbeiten zur Geschichte der Medizin, Heft 39).
30. Diastase was discovered in blood by M. Bial (1892), (Über die diastatische Wirkung des Blut- und Lymphserums, Pflügers Arch. Ges. Physiol. *52*, 137. (Vgl. auch Bial, M. (1893), Pflügers Arch. Ges. Physiol. *53*, 156)).
The detection in urine was published by Cohnheim, P. (1863), Zur Kenntnis der zuckerbildenden Fermente, Virch. Arch. Pathol. *28*, 241 and Béchamp, A. (1865) Sur la matière albuminoide-ferment de l'urine, C. R. Acad. Sci. *60*, 445–447.
Pepsin was discovered in urine by E. Brücke (1861), Beiträge zur Lehre von der Verdauung; Sitzungsberichte der K. Akademie der Wissenschaften in Wien *43*, 601; in blood it was detected many years later (van Calcar, R. P. (1912), Über die physiologisch-pathologische Bedeutung der weißen Blutkörperchen; Pflügers Arch. Ges. Physiol. *148*, 257).
31. Wohlgemuth, J. (1910), Beitrag zur funktionellen Diagnostik des Pankreas. Berliner Klin. Wochenschr. *47*, 92–95.
In 1908 *Wohlgemuth* had published a very careful experimental study (Über eine neue Methode zur Bestimmung des diastatischen Ferments, Biochem. Z. *9*, (1908) 1–9) which included a quantitative method for the determination of diastase activity. This method was based on the decrease of the iodine-starch reaction and was applicable to blood, urine, juice from the duodenum, feces, etc. In the following year he found in experiments with dogs that diastase in blood is raised after ligature of ductus pancreaticus. (Biochem. Z. *21* (1909), 381–422). Then he made similar observations in two clinical cases; Therefore in 1910 he proposed this diastase method as a clinical test for pancreas function.
32. *Peter Rona* (1871–1949) was director of the Chemical Department at the Hospital Am Urban in Berlin from 1906 to 1922. In 1922, he succeeded *E. Salkowski* as a director of the Chemical Department of the Institute for Pathology at Berlin University. In 1933 he was dismissed. (Biography: Ammon, R. (1960), In memoriam Peter Rona, Arzneimittelforsch. *10*, 321–327 (Portrait)).
33. Rona, P. & Michaelis, L. (1911), Über Ester- und Fettspaltung im Blute und im Serum. Biochem. Z. *31*, 345–354.
Instead of the Stalagmometer, introduced by Isidor Traube (1860–1943) (Ber. Dtsch. Chem. Ges. *20* (1887) 2644)), Rona and Michaelis proposed, for simplification of their method, the use of an ordinary pipet (see fig. 2).
34. Rona, P., Petow, H. & Schreiber, H. (1922), Eine Methode zum Nachweis blutfremder Fermente im Serum (Ein Beitrag zur Diagnose von Organerkrankungen) Klin. Wochenschr. *48*, 2366–2367. See also l. c. (35).
35. Rona, P. & Pavlović, R. (1932), Über die Wirkung des Chinins und des Atoxyls auf Pankreaslipase. Biochem. Z. *134*, 108–117. Citation from p. 108.
36. *Gerhard Katsch* in a plenary lecture („Zur Klinik der Pankreaserkrankungen") at the 4th meeting of the Gesellschaft für Verdauungs- und Stoffwechselerkrankungen at Berlin (22.–26. 10. 1924) coined the term „Fermententgleisung": „Bei irgendwelcher Abflußbehinderung, Kompression oder Obturation des *Wirsung*'schen Ganges kommt es nun zur Fermententgleisung ins Blut und zu vermehrter Ausscheidung im Harn". In the beginning *Katsch* did not refer to the acute inflammation of pancreas. This was included in the following year (see: Katsch, G. (1925), Die Diagnose der leichten Pankreatitis, Klin. Wochenschr. *4*, 289–293).
37. Garrod, A. E. (1908), Lancet *II*, 1, 73, 142, and 214, and the monograph published the following year: Garrod, A. E. (1909), Inborn Errors of Metabolism, Oxford.
38. The assumption, that certain inherited diseases of metabolism are caused by the lack of a particular enzyme was discussed occasionally, but the experimental proof was lacking until the nineteen-fifties onwards.
39. Schmidt, A. (1872), Neue Untersuchungen über die Faserstoffgerinnung. Pflügers Arch. Ges. Physiol. *6*, 413.
40. Quick, A. J. (1935), The prothrombin in hemophilia and obstructive jaundice. J. Biol. Chem. *109*, LXXIII. The short abstract presents a classical description of a coupled enzyme test: "On the assumption that blood clotting proceeds in two steps:
Prothrombin + thromboplastin + Ca = thrombin; fibrinogen + thrombin = fibrin and the rate of clotting is proportional to the concentration of thrombin, a means for the determination of prothrombin is proposed. If the first phase proceeds according to the law of mass action, the rate of thrombin formation is a product of the concentration of

prothrombin, thromboplastin, and Ca. When oxalated plasma is used and recalcified with the optimal amount of Ca, and an excess of thromboplastin added, only prothrombin is left as a variable and its concentration should determine the clotting time".

41. *Robert Robison* (1883–1941) was a pupil of *Arthur Harden* at the Lister Institute for Preventive Medicine in London and later at the same institute director of the chemical department. His work on phosphatase is related to his work on glucose-6-phosphate ("Robison-Ester") (Robison, R. (1922), Biochem. J. *16*, 809–824).
42. Robison, R. (1923), The possible significance of hexosephosphoric esters in ossification. Biochem. J. *17*, 286–293.
43. *Herbert Davenport Kay* (born 1893) was coworker of *R. Robison* (see Biochem. J. *18* (1924) 755) and became biochemist at the London Hospital in 1925. Later he was appointed Professor of Biochemistry at Toronto and became finally director of the National Institute for Research in Dairying at Reading, UK.
The first paper on plasma phosphatase in bone diseases was published in 1929: Kay, H. D. (1929), Plasmaphosphatase in osteitis deformans and in other diseases of bone. Brit. J. Exp. Pathol. *10*, 253–256. See also: Kay, H. D. (1930), Plasma phosphatase II. The enzyme in disease, particularly in bone disease. J. Biol. Chem. *89*, 249–266.
44. Kay, H. D. (1930), Plasma phosphatase I. Method of determination. Some properties of the enzyme. J. Biol. Chem. *89*, 235–247.
45. The increase of alkaline phosphatase in bile duct obstruction was described firstly by Roberts, W. M. (1930), Brit. J. Exp. Pathol. *11*, 90 (and Brit. Med. J. *1933*, 734).
The first methods for determination of activity used β-glycerophosphate as substrate (Kay, H. D. (1930), J. Biol. Chem. *89*, 235–247; Bodanski, A. (1933), J. Biol. Chem. *101*, 93–104).
King, E. J. & Armstrong, A. R., introduced phenylphosphate (Canad. Med. Assoc. J. *31*, (1934), 376–381). Later phenolphthalein phosphate (Huggins, C. & Talalay, P. (1945), J. Biol. Chem. *159*, 399) and *p*-nitrophenylphosphate (Bessey, O. A., Lowry, O. H. & Brock, M. J. (1946), J. Biol. Chem. *164*, 321–329), were used.
46. See the review by Ammon, R. & Chytrek, E. (1939), Die Bedeutung der Enzyme in der klinischen Diagnostik, Ergeb. Enzymforsch. *8*, 91–134.
47. Phosphatase with different pH optimum:
Bamann, E. & Riedel, E. (1934), Hoppe-Seyler's Z. Physiol. Chem. *229*, 125–150.
Acid phosphatase in prostatic tissue: Kutscher, W. & Wolbergs, H. (1935), Hoppe-Seyler's Z. Physiol. Chem. *236*, 237–240.
48. Synthesis of acid phosphatase in cell of prostatic carcinoma: Gutman, A. B., Sproul, E. E. & Gutman, E. B. (1936), Amer. J. Cancer *28*, 485.
49. Gutman, A. B. & Gutman, E. B. (1938), J. Clin. Invest. *17*, 473–478.
50. See the review by Ammon & Chytrek, l. c. (46).
51. Abderhalden, E. (1909), Hoppe-Seyler's Z. Physiol. Chem. *61*, 200.
52. Rothschuh, K. E. & Bleker, J. (1972), Die Einführung naturwissenschaftlich-messender Methoden in die klinische Diagnostik in der deutschen Medizin des 19. Jahrhunderts. Proc. 23rd Internt. Congress History of Medicine, London, 131–135.
cf. Shryock, R. H. (1961), The history of quantification in medical science, Isis *53*, 215–237.
53. Schwann, T. (1839), Mikroskopische Untersuchungen über die Übereinstimmung in der Struktur und dem Wachstum der Thiere und Pflanzen, Berlin.
54. Hoppe-Seyler, F. (1883), Handbuch der physiologisch und pathologisch-chemischen Analyse, 5th ed., Berlin.
55. Farber, E. (1961), Early studies concerning time in chemical reactions. Chymia *7*, 135–148.
56. Wilhelmy, L. (1850), Über das Gesetz, nach welchem die Einwirkung der Säuren auf den Rohrzucker stattfindet. Pogg. Ann. *81*, 413–433, 499–526.
57. Berthelot, M. (1862), Ann. Chim. Phys. *66*, 110–128.
58. Bidder, F. & Schmidt, C. (1852), Die Verdauungssäfte und der Stoffwechsel, G. A. Reyher Mitau – Leipzig.
59. Ostwald, W. (1902), Über Katalyse. Physikal. Zeitschr. *3*, 313–322.
60. O'Sullivan, C. & Tompson, F. W. (1890), Invertase: a contribution to the history of an enzyme or unorganized ferment. J. Chem. Soc. *57*, 834–930.
61. Brown, A. (1902), J. Chem. Soc. *81*, 373–388.
62. Henry, V. (1903), Lois générales de l'action des diastases; Hermann, Paris.
63. Michaelis, L. & Menten, M. L. (1913), Die Kinetik der Invertinwirkung. Biochem. Z. *49*, 333–369.
A short biography of *L. Michaelis* is given by Scheuch, D. W. (1979), Zur Erinnerung an Leonor Michaelis, Z. Med. Laboratoriums-Diagnostik *20*, 252–256 (with a portrait).
64. a. Report of the Commission of Enzymes of the International Union of Biochemistry, London, 1961.
b. King, E. J. & Campbell, D. M. (1961), Clin. Chim. Acta *6*, 301.
65. Kohler, R. E. (1973), The enzyme theory and the origin of biochemistry, Isis *64*, 181–196.
66. From: Michaelis, L. (1921), Praktikum der physikalischen Chemie, insbesondere der Kolloidchemie für Mediziner und Biologen. J. Springer, Berlin.
67. Schrader, J. C. C. (1811/2), Über die neue von Kirchhof(f) entdeckte Zuckergewinnung. J. für Chemie u. Physik (Halle) *4*, 108–110.
68. Davy, H. (1817), Phil. Transactions *1817*, 77–85.
69. Thénard, L. J. (1818), Ann. Chim. Phys. (Paris) *8*, 306 und *9*, 314, 441.
70. Döbereiner, J. W. (1823), Über neuentdeckte, höchst merkwürdige Eigenschaften des Platins und die pneumatisch-capillare Tätigkeit gesprungener Gläser, Jena.
71. Mitscherlich, E. (1834), Ann. Phys. Chem. *31*, 273.
72. Tiedemann, F. & Gmelin, L. (1826), Die Verdauung nach Versuchen. Heidelberg, Groos.
73. Beaumont, W. (1833), Experiments and observations on the gastric juice, and the physiology of digestion. Plattsburgh, Allen.
74. Eberle, J. N. (1834), Physiologie der Verdauung nach Versuchen auf natürlichem und künstlichem Wege. Würzburg.
75. Schwann, Th. (1836), Arch. Anat. *1836*, 90–138.
76. Frerichs, F. Th. (1846), Die Verdauung. In: Handwörterbuch der Physiologie mit Rücksicht auf physiologische Pathologie, Bd. 3, 658–872, Braunschweig.
77. Liebig, J. (1839), Liebigs Ann. Chem. Pharm. *30*, 250–288.
78. v. Nägeli, C. (1879), Theorie der Gärung. Ein Beitrag zur Molekularphysiologie. München, Oldenbourg.
79. Traube, M. (1858), Ann. Physik *103*, 331–344.
80. Fischer, E. (1894), Ber. Dtsch. Chem. Ges. *27*, 2985–2993.
81. Kirchhoff, C. G. S. (1814), J. für Chemie u. Physik *14*, 389–398.
82. Payen, A. & Persoz, J. F. (1833), Ann. Chim. Phys. *53*, 73–92.
83. Meyer, K. H., Fischer, E. H., Staub, A. & Bernfeld, P. (1948), Helv. Chim. Acta *31*, 2158–2164.
84. Northrop, J. H. (1930), J. Gen. Physiol. *13*, 739–766. Cf. Northrop, J. H. (1932), Story of isolation of crystalline pepsin and trypsin, Scientific Monthly *35*, 333–340.
85. Robiquet, P. J. & Boutron-Chalard, A. F. (1830), Ann. Chim. Phys. *44*, 352.
86. Dubrunfaut, A. P. (1846), Ann. Chim. 3^{me} ser. *18*, 99–108.
87. Berthelot, M. (1860), C. R. Acad. Sci. (Paris) *50*, 980–984.
88. Musculus, F. A. (1876), C. R. Acad. Sci. (Paris) *82*, 334.
89. Corvisart, L. (1857), Collection de mémoires sur une fonction peu connue du pancreas, la digestion des aliments azotés. Paris.
90. Kühne, W. (1877), Verh. Naturh.-Med. Verein Heidelberg, N. F. *1*, 194–98.
91. Northrop, J. H. & Kunitz, M. (1931), Science *73*, 262. Cf. l. c. (84).
92. Hughes, G. (1750), Natural History of Barbados.
93. Wurtz, A. & Bouchut, E. (1879), C. R. Acad. Sci. (Paris). *89*, 425.

94. Balls, A. K. & Lineweaver, H. (1939), J. Biol. Chem. *130*, 669–686.
95. Gutman, E. B. & Gutman, A. B. (1940), J. Biol. Chem. *136*, 201–209.
96. Abul-Fadl, M. A. M. & King, E. J. (1949), Biochem. J. *45*, 51–60.
97. Fishman, W. H. & Lerner, F. (1953), J. Biol. Chem. *200*, 89.

Prof. Dr. Dr. J. Büttner
Institut für Klinische Chemie
Medizinische Hochschule Hannover
Karl-Wiechert-Allee 9
D-3000 Hannover 61